Dietary intake and micronutrient status of adolescents: effect of vitamin and trace element supplementation on indices of status and performance in tests of verbal and non-verbal intelligence

BY SUSAN SOUTHON, A. J. A. WRIGHT, P. M. FINGLAS, ANGELA L. BAILEY, JULIE M. LOUGHRIDGE AND A. D. WALKER

Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA

(*Received 7 July 1992 – Revised 11 November 1993 – Accepted 15 November 1993*)

Relationships between micronutrient intake and status, and micronutrient status and performance in tests of intelligence were investigated in a group of adolescents (13–14 years old). Dietary intakes were assessed using a 7 d weighed dietary record method, coupled with the collection of duplicate diets. Vitamin and trace mineral intakes calculated using food composition tables were compared with those obtained by direct analysis of duplicate diets. Micronutrient status was judged via a range of biochemical indices measured in blood samples taken after a 12–15 h fast. Blood samples were taken both before and after a 16-week period of vitamin and trace mineral supplementation. Individual tests of verbal and non-verbal intelligence were also performed pre- and post-supplementation. The results of this study indicate that the use of food table data may lead to substantial over- or underestimation of the intake of several micronutrients. In general, the total calculated or analysed amount of a specific micronutrient consumed did not adequately predict status, as judged by a range of biochemical indices. There were significant changes in status measurements over the 16-week study period, irrespective of supplementation, and these changes were markedly influenced by the initial status of the subject. There was no effect of supplementation on performance in tests of intelligence. However, there was a significant association between plasma ascorbic acid and initial non-verbal intelligence quotient (IQ) in the boys, and between whole blood glutathione peroxidase (*EC* 1.11.1.9) activity and non-verbal and verbal IQ in both sexes. These findings are discussed in relation to other recent studies of the influence of micronutrient supplementation on the psychological performance of children.

Micronutrient supplementation: Nutrient status: Intelligence: Adolescents

In the UK, with improved standards of living and more equitable distribution of food, overt nutritional deficiencies have been virtually eradicated (Department of Health and Social Security, 1978) but the extent and impact of subclinical or 'marginal' deficiency states is largely unknown. Recently, there has been speculation about the potential adverse effect of marginal micronutrient deficiency on the intellectual performance of children. This speculation arose as a result of two studies (Benton & Roberts, 1988; Benton & Buts, 1990) which showed an improvement in tests of non-verbal intelligence of schoolchildren after 8 and 4 month periods of combination micronutrient supplementation respectively. However, in the second of these studies (Benton & Buts, 1990) a significant positive effect of supplementation was apparent only in a subgroup of boys who were assessed as having 'poorer' diets as determined by the number of times vitamin and mineral intakes fell below 50% of the recommended daily amount (RDA), when dietary intake was assessed using a 15 d dietary diary method. Two further studies of the influence of vitamin–mineral supplementation on the non-verbal intelligence quotient (IQ) of schoolchildren were

performed by other workers. Nelson *et al.* (1990) provided a supplement for 4 weeks, whilst Crombie *et al.* (1990) supplemented for 7 months. Both of these studies failed to find a significant difference between supplemented and placebo groups in tests of intelligence. The most recent, and largest, study was performed by Schoenthaler *et al.* (1991*b*). Results presented in this study were interpreted as providing further evidence of 'highly significant' improvements in non-verbal IQ resulting from supplementation of the diet of school-children with vitamins and minerals. A full analysis of the data and research methods used in the above studies is beyond the scope of this paper and has been dealt with extensively elsewhere (*Lancet* letters, 1988*a*, *b*; Blinkhorn, 1991; Eysenck, 1991), but one possible reason for the discrepancy between the studies discussed above is that subjects who showed no response to supplementation were better nourished (Schoenthaler *et al.* 1991*b*). However, none of the above investigations presented information on the nutritional status of their subjects. It cannot be assumed that vitamin and mineral intake, calculated from tables of food composition, reflects either the actual intake, or the micronutrient status, of the individual being assessed. Determination of true dietary intake is problematic (Bingham, 1987) and the suitability of food table data for the calculation of intake for individuals is questionable. It is also well known that for several micronutrients the total amount consumed may bear little relationship to the amount absorbed, because of a range of physiological and dietary factors that markedly influence bioavailability (Southon *et al.* 1988; Schrijver, 1991). In addition, there is insufficient information available on the relationship between nutrient intake and body status in young, growing individuals to be able to make sound judgements on the micronutrient status of children based solely on calculated intakes (Southon *et al.* 1992), or on the influence of short-term, moderate dietary supplementation on status.

The present paper is concerned with:

(1) The examination of the reliability of dietary assessments of nutrient intake when assessment is based on data calculated from food tables; (a) Is the 7 d average daily intake of nutrients as calculated from food tables statistically different from the 7 d average intake of nutrients as assessed by the direct analysis of duplicate weighed diets? (b) Is the calculated intake for each nutrient significantly correlated to analysed intake?

(2) The examination of the reliability of assessments of nutrient intake to predict micronutrient status; is there a relationship between 7 d average nutrient intake and biochemical indices of status?

(3) The examination of the response of various biochemical indices of micronutrient status to moderate vitamin and trace mineral supplementation.

(4) The examination of the relationship between biochemical indices of micronutrient status and performance in tests of verbal and non-verbal intelligence in a group of young adolescents; (a) Is IQ related to initial micronutrient status before treatment with supplement or placebo? (b) Does supplementation improve IQ, particularly the non-verbal IQ score, as suggested by some other workers? (c) If there are changes in either micronutrient status and/or IQ over the treatment (supplement or placebo) period, is there any relationship between change in status and change in IQ?

In the present study the dietary intake of 13–14-year-old boys and girls was assessed by a 7 d weighed record method, coupled with the direct analysis of duplicate diets. Following dietary assessment, a combination trace mineral and vitamin supplement was administered daily for 16 weeks. Relationships between calculated and analysed nutrient intake, intake and biochemical status for selected micronutrients, and status and performance in tests of intelligence, both pre- and post-supplementation, were statistically examined.

MATERIALS AND METHODS

Subjects

The study was approved by the Institute of Food Research's Ethics Committee before recruitment.

Subjects, 13–14 years, were recruited from two local-authority schools. Sixty-one subjects were recruited. Seven subjects withdrew before commencing the study leaving a total of fifty-four subjects, thirty-five girls and nineteen boys. The median (range) age, height and weight of the girls and boys respectively was: age 13·8 (13·3–14·3), 13·6 (13·2–14·3) years; height 1·584 (1·503–1·737), 1·616 (1·423–1·866) m; weight 51·2 (38·5–62·5), 46·7 (33·1–68·0) kg. Median heights and weights for the subjects were similar to the 50th percentile values calculated by the National Centre for Health Statistics for this age group (Thomas, 1988). None of the subjects was on medication during the study period and none admitted to being habitual smokers.

Measurement of intake

Each subject kept a written, weighed record of everything they ate and drank every 6th day for 7 weeks (Black *et al.* 1984) between 1 November and 21 December 1989. Dietary records were coded using *McCance and Widdowson's The Composition of Foods* (Paul & Southgate, 1978), together with the *Immigrant Food Supplement* (Tan *et al.* 1985), *Additional Foods* (Wiles *et al.* 1980), *Cereals and Cereal Products* (Holland *et al.* 1988) and *Milk Products and Eggs* (Holland *et al.* 1989). Mean daily nutrient intakes were calculated using the Institute of Food Research food composition database.

On each of the days of dietary recording the subjects also collected duplicate portions of all food and drink consumed. Duplicate meals were kept overnight at −18° in a domestic freezer until they were collected early the following day, along with dietary record sheets. The records were checked in the presence of the subject to ensure that all the necessary detail had been provided. Duplicate diets were returned to the laboratory where they were homogenized; distilled water was added to aid homogenization if necessary. The weight of any water added and the weight of the homogenized diet was recorded. Subsamples (approximately 100 g) were taken into tightly capped, acid-washed, plastic containers and stored at −40° until analysed. One sample was taken for immediate total ascorbate analysis.

A detailed description of the methods used to collect the weighed records of food intake and duplicate diets is presented by Finglas *et al.* (1993).

Duplicate diet analysis

The following analyses were performed:

Iron, zinc and copper. Freeze-dried samples (approximately 5 g) of homogenized diet were accurately weighed into silica crucibles, dry ashed at 480° for 48 h and taken up into concentrated (11 M) HCl for Fe, Zn and Cu analyses by atomic absorption spectrometry (PU 9000; Pye Unicam, Cambridge). Analyses were performed in duplicate on 378 individual days' diets.

Vitamin C. Total ascorbate was determined by a HPLC technique. The method used was essentially that of Behrens & Madere (1987) with slight modification and is described in detail elsewhere (Finglas *et al.* 1993). A total of 378 diets were analysed in duplicate.

Thiamin. Duplicate samples (10 g) of homogenized diet were extracted for the determination of total thiamin using the HPLC method described by Bailey & Finglas

(1990) with the following modification: the stock oxidizing agent prepared was 0·30 M- rather than 0·03 M- $K_3Fe(CN)_6$. This modification was found to improve the oxidation of thiamin to thiochrome in mixed diet samples during the extraction procedure. Thiamin analysis was performed on combined samples of homogenized diet. Combined samples were prepared by thoroughly mixing 5 g/kg subsamples of each of the seven duplicate diets collected from one individual. Fifty-four combined samples were analysed for thiamin and for each of the following vitamins.

Riboflavin. Duplicate samples (10 g) of the 7 d combined diets were analysed for riboflavin content using the HPLC method described by Kwiatkowska *et al.* (1989), with the following modifications to the chromatographic conditions. The HPLC column used was an Apex ODS 3 μm, 150 mm × 4·6 mm i.d. (Jones Chromatography Ltd., Hengoed, Mid Glamorgan) with reversed-phase C18 10 μm guard column (Anachem Ltd., Luton, Beds.). The mobile phase was methanol–water (1:1 v/v) pumped at a flow rate of 0·5 ml/min. The retention time for riboflavin was 4·75 min.

Vitamin B_6. The total B_6 content of duplicate subsamples (10 g) of homogenized, combined 7 d diets was determined using the method of Brubacher *et al.* (1985) with the following modifications. Samples were autoclaved for 20 min with 25 ml 0·2 M-H_2SO_4 and after cooling the pH was adjusted to 4·5 using NaOH. Samples were incubated at 45–50° for 2 h with amyloglucosidase (*EC* 3.2.1.3; 10 mg/ml), to assist extraction, cooled and made to volume (50 ml) with distilled water. A Spherisorb® ODS 5 μm column (250 mm × 4·6 mm i.d.; Anachem Ltd.) was used. The mobile phase was 0·04 M-H_2SO_4–methanol (99:1, v/v) and the flow rate was 1·5 ml/min. Retention times were 2·15, 5·47 and 7·97 min for pyridoxamine, pyridoxal and pyridoxine respectively.

Pteroylglutamates. Duplicate subsamples (1 g) of homogenized, combined 7 d diets were deconjugated and assayed microbiologically (Phillips & Wright, 1983) with *Lactobacillus rhamnosus* NCFB 243 by the method of aseptic addition (Herbert, 1966) at pH 6·2 (Phillips & Wright, 1982). Deconjugation of pteroylglutamates was performed using human plasma (Lakshmaiah & Ramasastri, 1980) in the presence of other digestive enzymes to assist release (De Souza & Eitenmiller, 1990). Duplicate samples were added to 8·5 ml freshly prepared 0·1 M-potassium phosphate buffer containing 57 mmol/l ascorbic acid (pH 6·0). Pancrex V (Paynes and Byrne Ltd., Greenford, Middlesex) solution (0·1 ml; 2 capsules/10 ml distilled water) and human plasma (0·4 ml; 5 ml freeze-dried plasma taken up into 20 ml 1 M-L-cysteine–HCl to stabilize conjugase activity; Sigma, Poole, Dorset) were added. The mixture was incubated at 37° for 2 h and then at 100° for 20 min. Deconjugated extracts were made up to 25 ml with phosphate buffer, mixed, centrifuged (2000 ***g***, for 15 min) and the supernatant was stored at −20°. Diluted samples (50 μl) were analysed in triplicate on microtitration plates (Tamura, 1990). A calibration series of pteroylmonoglutamic acid (0–100 pg/well) was included on each plate.

Total energy. A subsample of freeze-dried combined 7 d duplicate diets was taken and the heat of combustion of 0·5–1·0 g dry matter was determined in an adiabatic bomb calorimeter (Gallenkamp, Loughborough, Leics.) using benzoic acid as a thermochemical standard. Metabolizable energy (ME) was calculated from the combustible (bomb) energy (BE) using the formula of Miller & Payne (1959):

$$\text{ME kJ (mean/d)} = (\text{BE kJ (mean/d)} \times 0{\cdot}95) - (\text{N (mean g/d)} \times 30 \text{ kJ}).$$

Blood collection and preparation

Fasting (12 h) venous blood samples (approximately 12 ml) were obtained between 9 November and 7 December during the period of dietary assessment but not on, or

immediately following, a day of dietary recording. Part (1·5 ml) of the unheparinized, whole-blood sample was taken for serum ferritin analysis. Serum was separated by centrifugation at 8800 ***g*** for 4 min within 2 h of collection and the serum was stored at −40° until analysed. The remainder of the blood (approximately 10 ml) was dispensed into a heparinized tube. A sample (1·5 ml) of fresh (within 1 h of collection), whole, heparinized blood was centrifuged at 8800 ***g*** for 4 min, the plasma separated, placed on ice for 10 min and then analysed for ascorbic acid. A further sample (100 μl) of well-mixed, heparinized whole blood was taken for haemoglobin (Hb), packed cell volume (PCV) erythrocyte count (RBC), mean cell volume (MCV) and leucocyte count (WBC) determinations, which were performed within 4 h of collection. Other samples of heparinized whole blood were taken for glutathione peroxidase (*EC* 1.11.1.9; 100 μl) and pteroylmonoglutamate (100 μl) assay. The remaining heparinized blood was centrifuged at 2500 ***g*** for 10 min at 4°, the plasma was removed and subsamples were taken for measurement of alkaline phosphatase (*EC* 3.1.3.1) activity (200 μl) and measurement of Zn and Cu (2 ml), pyridoxine (500 μl), cyanocobalamin and pteroylmonoglutamate (500 μl), vitamin D (100 μl), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triacylglycerol (1 ml) concentrations. Remaining packed erythrocytes were thoroughly washed with an equal volume of cold (4°), isotonic saline (9 g NaCl/l) and centrifuged at 2500 ***g*** for 10 min at 4°. The washing procedure and centrifugation were repeated once more and the resulting packed cells were resuspended in isotonic saline. Subsamples (1 ml) were taken for determination of erythrocyte superoxide dismutase (*EC* 1.15.1.1), transketolase (*EC* 2.2.1.1) and glutathione reductase (*EC* 1.6.4.2) activities, and total thiamin concentration. Samples were centrifuged at 8800 ***g*** for 4 min and the supernatant was removed. Packed cells for measurement of erythrocyte transketolase (ETK) and erythrocyte glutathione reductase (EGR) activity were resuspended in distilled water.

All the above subsamples, with the exception of that for alkaline phosphatase analysis, were placed immediately on dry ice and stored at −196° (samples for measurement of ETK and EGR activity) or −40° (all other samples) until analysed. Plasma used for the determination of alkaline phosphatase activity was placed at 4° and analysed within 4 h of collection.

A second fasting blood sample was taken two clear days after the period of placebo or vitamin and mineral supplementation.

Blood analysis: indices of micronutrient status

Iron. Hb, PCV, RBC, MCV and WBC were measured using a semi-automated Coulter Counter (model CBC-5; Coulter Electronics, Luton, Beds) using Coulter's '4C-normal cell control' as quality control.

Serum ferritin was determined using an ELISA procedure (Enzymum-Test Ferritin kit; Boehringer Mannheim Immunodiagnostics, Mannheim, Germany).

Zinc and copper. Samples (2 ml) of deproteinized plasma were analysed for Zn and Cu by atomic absorption spectroscopy.

Plasma alkaline phosphatase activity was determined colorimetrically at 25° using a kit assay (Test-combination alkaline phosphatase kit; Boehringer Mannheim Diagnostica, Mannheim, Germany). To convert to assay units at 37°, multiply tabulated values by a factor of 1·6.

Erythrocyte superoxide dismutase activity was measured using a kit assay (RANSOD; Randox Laboratories Ltd., Crumlin, County Antrim).

Selenium. Whole-blood glutathione peroxidase activity was measured using the modified method of Paglia & Valentine (1967), as described by Whanger *et al.* (1988).

Ascorbic acid. Plasma ascorbic acid (PAA) concentration was determined using a HPLC technique (Finglas *et al.* 1993), except that plasma samples were stabilized with an equal volume of metaphosphoric acid (60 g/l).

Cholecalciferol. Plasma 25-hydroxycholecalciferol was measured using a radioimmunoassay procedure (25-OH D ^{3}H RIA kit; INCSTAR Corp., Stillwater, MN, USA).

Thiamin. Total erythrocyte thiamin was measured using the method of Bailey & Finglas (1990) with the following modifications. Samples (0·5 ml) of erythrocytes were extracted with 1·5 ml 0·133 M-HCl by heating in a boiling water bath for 30 min. The enzyme suspension used was 10 mg/ml takadiastase (*EC* 3.2.1.1 and *EC* 3.2.1.2; a crude α- and β-amylase extract from *Aspergillus oryzae*) and 1 mg/ml acid phosphatase (*EC* 3.1.3.2) in 2 M-sodium acetate buffer, pH 4·5. The oxidizing agent was 0·5 mg/ml $K_3Fe(CN)_6$ in 2 M-sodium acetate buffer, pH 5·5, containing 25 mM-EDTA. These modifications were made to maximize recovery and sensitivity given the small volume of blood available for the assay.

ETK activity was measured using a method described by Anderson & Nicol (1986). The basal activity of the enzyme was measured followed by determination of activity with excess coenzyme (thiamin pyrophosphate) added *in vitro*. The data are expressed in terms of an activated coefficient (ETK Ac) calculated as follows:

$$\text{Ac} = \frac{\text{enzyme activity with added coenzyme}}{\text{basal enzyme activity}}$$

Riboflavin. Basal and stimulated (with added coenzyme; flavine adenine dinucleotide) erythrocyte glutathione reductase activities were determined (Powers *et al.* 1983) and used to calculate the activity coefficient (EGR Ac).

Cyanocobalamin and pteroylmonoglutamate. Plasma cyanocobalamin and pteroylmonoglutamate were measured using a radioassay kit (Quantaphase B_{12}/Folate radioassay; BIO-RAD Lab. Ltd, Watford, Herts).

Pyridoxine. Plasma pyridoxal-5-phosphate was measured by the method of Naoi & Ichinose (1988) with the following modifications. Plasma samples (100 μl) were diluted with 100 μl 10 mM-potassium phosphate buffer (pH 7·4) and extracted with 200 μl 100 g/l trichloroacetic acid. KCN (40 μl, 8 mM) was used to produce cyanide derivatives. The volume of all other reagents used was increased by approximately twofold.

Lipids. Plasma cholesterol was analysed using the Boehringer-Mannheim enzymic, colorimetric, 'Cholesterol C-System (CHOD-PAP)'. HDL was assayed using the same cholesterol kit after LDL and very-low-density lipoprotein precipitation using Boehringer-Mannheim's 'HDL Cholesterol' solution of phosphotungstic acid and Mg ions. Triacylglycerols were analysed using the Boehringer-Mannheim 'Triglyceride fully enzymatic UV method'. Precinorm-L (Boehringer-Mannheim) was used as a quality control for cholesterol, HDL and triacylglycerols. LDL was calculated in mmol/l using the equation:

$$\text{LDL} = \text{total cholesterol} - (\text{triacylglycerols}/2{\cdot}2) - \text{HDL-cholesterol}$$

as described by Friedewald *et al.* (1972).

IQ tests

All subjects underwent psychological testing using the Wechsler Intelligence Scale for Children – Anglicized Revised Edition (WISC-R UK). Each subject was interviewed individually both before (during the period of dietary recording and collection) and directly after (within 7 d) the 4-month placebo or supplement–treatment experiment. All tests were conducted by the same educational psychologist.

Supplementation

Fifty-one subjects (three subjects declining to take part) were allocated to one of two groups which were matched for age, sex, height, weight and overall IQ. One group was given a vitamin–trace mineral supplement, the other a mannitol-based placebo which was identical in appearance. Over the first few days of the supplementation period, four girls, who were allocated to the test group, reported slight nausea within 10–15 min after taking their capsules. These subjects were transferred immediately to the control group; both psychologist and analysts remained blind to this reallocation. This did not significantly alter the original group matchings: final group matchings are listed in Table 1. The composition of the supplement is given in Table 2. For technical manufacturing reasons, vitamins A, E and K could not be added to the other listed micronutrient components to form a single supplement and, as it was thought important to minimize the complexity of the supplementation regimen that subjects would have to undergo, a compromise had to be made and these micronutrients were omitted in favour of the addition of the maximum number of micronutrients that could be compatibly incorporated into a single vitamin–trace mineral supplement. The supplement was manufactured by R. P. Scherer Ltd, Swindon, Wilts. and is not commercially available. The supplement was prepared in capsule form and subjects were requested to take two capsules/d. Each capsule contained 50% of the UK RDA value (Department of Health and Social Security, 1979) for each vitamin and trace mineral included in the supplement, or 50% of the US recommended dietary allowance (National Research Council, 1980) where no UK value was given. No RDA value has been established for Se, Cr and Mn. However, none of the dose levels for these nutrients exceeded estimated safe and adequate daily dietary intakes (National Research Council, 1980). Minerals, other than the trace minerals, were excluded from the formulation because of the practical problems associated with incorporating them in appropriate amounts into the daily supplementation regimen. RDA were used as a basis for the formulation of the supplement, since currently available RNI (reference nutrient intake) values (Department of Health, 1991) had not been published at the time the study was performed.

Capsules were given to subjects weekly, in coded containers, by fieldstaff who were not informed as to the nature of the contents. Each subject was seen once weekly, either at school or at home, when a fresh supply of capsules was provided. If any capsules had not been taken, the number was recorded and totalled at the end of the study. No subject appeared to have taken less than 75% of the capsules supplied. Compliance in the treatment group was also assessed by comparing pre- and post-supplementation values for indices of status.

Statistical treatment of results

All descriptive data (height, weight, initial verbal and non-verbal scores), dietary intake data (calculated and analysed nutrient intake), status measurements (biochemical indices of mineral and vitamin status in blood) and IQ data, both before and after supplement or placebo treatment, were examined for normality of distribution and, if necessary, $\log_{10}$ transformed before any further statistical treatment. All data for two-way- or three-way-analysis of variance tests were examined for variance homogeneity and, if necessary, also $\log_{10}$-transformed before analysis. For three-way analysis of variance tests, where one variable was 'time', subject codes were 'blocked' so that the effects of treatment (supplement or placebo) over the experimental period could be examined within each subject. Possible differences between ranked data were assessed using the non-parametric U-test. Correlation coefficients for ranked data were obtained using Spearman's non-parametric rank-correlation test. The type of statistical analysis used is shown in each of the tables of results.

Table 1. *Values for height, weight, verbal and non-verbal intelligence quotient (IQ) test scores for adolescent subjects aged 13–14 years given a vitamin–mineral supplement (S) or a placebo (P)**

(Values are means with their standard errors† with ranges in parentheses)

Treatment...	Boys				Girls			
	P (*n* 9)		S (*n* 10)		P (*n* 20)		S (*n* 12)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Height (m)	1·62 (1·47–1·89)	0·04	1·60 (1·42–1·74)	0·03	1·61 (1·50–1·74)	0·01	1·59 (1·53–1·73)	0·02
Weight (kg)	45·9 (35·5–54·8)	3·3	48·9 (33·1–68·0)	3·4	50·8 (38·5–62·5)	1·7	51·2 (43·9–61·2)	1·6
Verbal IQ score	59 (39–80)	4	57 (48–70)	2	57 (37–75)	2	55 (37–71)	3
Non-verbal IQ score	55 (51–64)	1	57 (39–69)	3	60 (47–66)	1	58 (48–75)	2

* For details of subjects and supplements, see Table 2, p. 899 and pp. 902–904.
† There were no significant differences between groups on the basis of sex or treatment.

Table 2. *Nutrient composition of the vitamin and mineral supplement (per two capsules)*

Nutrient		Chemical form
Iron (mg)	12	Ferrous sulphate
Zinc (mg)	15	Zinc sulphate
Copper (mg)	2	Copper II sulphate
Manganese (mg)	2·5	Manganese II sulphate
Chromium (μg)	50	Chromium–amino acid chelate
Selenium (μg)	50	Selenium–amino acid complex
Iodine (μg)	150	Potassium iodide
Vitamin D (μg)	10	Ergocalciferol
Ascorbic acid (mg)	25	Ascorbic acid
Thiamin (mg)	1	Thiamin mononitrate
Riboflavin (mg)	1·4	Riboflavin
Nicotinic acid equivalents (mg)	16	Nicotinamide
Pyridoxine (mg)	1·8	Pyridoxine hydrochloride
Cyanocobalamin (μg)	3	Cyanocobalamin
Pteroylmonoglutamic acid (μg)	400	Pteroylmonoglutamic acid
Biotin (μg)	100	D-Biotin
Pantothenic acid (mg)	4	Calcium pantothenate

RESULTS

Calculated v. *analysed nutrient intakes*

Calculated and analysed mean daily nutrient intakes and the range of intake for each nutrient are presented in Table 3. The average energy intake approximated to the estimated average requirement (EAR) for this age-group and, in general, the intake of micronutrients was around or well in excess of the RNI (Department of Health, 1991). Two exceptions were average Fe and Zn intakes in girls which were below the EAR of 11·4 and 7·0 mg/d respectively, both on a calculated and an analysed basis. Calculated energy intake for the boys was significantly lower than the analysed value, but the difference was only in the

Table 3. *Calculated and analysed daily intake of nutrients for adolescent subjects (13–14 years old)*†

(Values are means with their standard errors, with ranges in parentheses)

	Boys (*n* 19)				Girls (*n* 32)			
	Calculated		Analysed		Calculated		Analysed	
Nutrient	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy (MJ)	8·83* (5·76–12·22)	0·36	9·25 (6·96–12·72)	0·35	7·23 (4·31–11·13)	0·26	7·36 (4·54–10·95)	0·24
Protein (g)	67·3 (48·1–95·3)	2·9	—	—	53·7 (32·6–86·3)	2·0	—	—
Fat (g)	82·1 (58·3–119·6)	4·0	84·8 (57·5–126·8)	4·4	71·0 (35·9–126·5)	3·4	69·5 (28·7–112·2)	3·2
Carbohydrate (g)	293·7 (184·7–427·7)	14·5	—	—	232·4 (151·0–318·6)	7·9	—	—
Dietary fibre (g)	22·9 (14·1–45·4)	1·8	—	—	17·9 (7·9–27·9)	1·0	—	—
Calcium (mg)	899 (130–1315)	60	—	—	761 (325–1242)	41	—	—
Iron (mg)	14·9 (7·5–55·5)	2·4	13·2 (7·6–25·5)	1·0	9·7 (5·7–16·4)	0·5	9·8 (5·2–16·7)	0·5
Zinc (mg)	9·3 (6·5–14·5)	0·6	9·4 (6·2–14·3)	0·5	6·8 (1·1–14·1)	0·4	6·6 (4·0–9·2)	0·3
Copper (mg)	1·62 (0·95–2·45)	0·10	1·36 (0·78–3·41)	0·16	1·24*** (0·75–2·17)	0·16	0·87 (0·58–1·36)	0·03
Thiamin (mg)	1·51* (0·75–3·55)	0·16	1·95 (0·97–4·49)	0·19	1·08*** (0·56–1·80)	0·05	1·54 (0·64–2·73)	0·1
Riboflavin (mg)	1·97 (0·90–3·69)	0·18	1·80 (0·75–4·24)	0·18	1·34* (0·36–2·66)	0·08	1·23 (0·37–2·28)	0·07
Nicotinic acid equivalents (mg)	32·4 (18·7–51·0)	2·1	—	—	25·3 (14·8–43·8)	1·0	—	—
Pyridoxine (mg)	1·26 (0·75–1·87)	0·07	1·04 (0·39–2·50)	0·11	1·19*** (0·66–1·56)	0·04	0·65 (0·36–1·35)	0·04
Ascorbic acid (mg)	121 (24–428)	22	121 (49–246)	13	75 (19–182)	7	84 (43–153)	5
Vitamin E (mg)	5·4 (2·9–8·2)	0·3	—	—	5·0 (1·2–9·1)	0·3	—	—
Cyanocobalamin (μg)	4·0 (1·8–12·5)	0·7	—	—	2·6 (0·8–9·5)	0·3	—	—
Pteroylglutamate (μg)	174*** (91–318)	14	332 (89–625)	33	145*** (61–278)	8	252 (96–510)	19
Vitamin D (μg)	1·6 (0·7–3·5)	0·2	—	—	2·1 (0·2–7·2)	0·3	—	—
Retinol equivalents (μg)	952 (306–2882)	172	—	—	684 (130–235)	79	—	—
Biotin (μg)	17·1 (2·0–28·4)	1·4	—	—	13·2 (5·4–24·5)	0·8	—	—

Calculated intake was significantly different from analysed intake: * $P < 0.05$, *** $P < 0.001$ (Student's paired *t* test).

— No data obtained.

† For details of subjects and procedures, see Table 1 and pp. 899–900.

order of 4%. There was no significant difference between calculated and analysed energy intakes for girls.

There were several significant differences (Student's paired *t* test) between 7 d mean calculated and analysed intakes for the micronutrients. For both boys and girls, average pteroylglutamate intake calculated from tables was in excess of 40% lower than the analysed value. Calculated thiamin intake was also significantly lower (by between 23 and 27%) than the analysed value for both sexes. On the other hand, calculated Cu, riboflavin and pyridoxine intakes for girls were significantly higher than the values obtained by direct analysis and a similar trend was observed for boys, but differences just missed significance at $P < 0{\cdot}05$. Average calculated intakes for Fe, Zn and vitamin C were not significantly different from analysed values in either sex.

There was a significant correlation between calculated and analysed intake data, for both sexes, for energy (r 0·9; $P < 0{\cdot}001$), Fe (r 0·8; $P < 0{\cdot}001$), Zn (r 0·89; $P < 0{\cdot}001$), Cu (r 0·6; $P < 0{\cdot}01$), thiamin (boys, r 0·6; girls r 0·4; $P < 0{\cdot}01$), riboflavin (r 0·8; $P < 0{\cdot}001$) and vitamin C (boys, r 0·8; girls r 0·7; P 0·001). There was no relationship between calculated and analysed pteroylglutamate intake for boys and the relationship for girls was poor (r 0·4; $P < 0{\cdot}05$). There was no relationship between calculated and analysed pyridoxine intake in either sex. Further details of these analyses are presented by Southon *et al.* (1992) and Finglas *et al.* (1993).

Micronutrient intake v. *biochemical indices of status and the effect of supplementation*

Values for a range of biochemical indices of vitamin and trace mineral status are presented in Tables 4 and 5 respectively. Results of statistical analysis (three-way analysis of variance) are shown in Table 6. The percentages of boys and girls respectively with 'low' indices of status were as follows: serum ferritin (< 10 μg/l) 11 and 21; plasma Cu (< 11·0 μmol/l) 29 and 62; plasma pyridoxal-5-phosphate (< 34·4 nmol/l) 50 and 23; ETK-Ac (> 1·14) 22 and 35; and EGR-Ac (> 1·20) 37 and 31. Values for other indices of status were within the normal range. Consideration of reference limits and cut-off points for biochemical indices of micronutrient status is included in the Discussion section.

A significant correlation between calculated intake and status was found only for vitamin C *v.* plasma ascorbic acid (PAA) in boys (r 0·63, $P < 0{\cdot}001$) and riboflavin *v.* EGR-Ac in girls (r 0·33, $P < 0{\cdot}05$). Using analysed intake values, a significant correlation was found between vitamin C intake and PAA in both sexes (boys, r 0·59, $P < 0{\cdot}01$; girls, r 0·33, $P < 0{\cdot}05$) and between pyridoxine intake and plasma pyridoxal phosphate in boys (r 0·56, $P < 0{\cdot}05$).

There was an almost ubiquitous effect of time (Ti) on the indices of status measured (Table 6), which in many cases was independent of a treatment (Tr) effect. Mean values for PCV, MCV, alkaline phosphatase activity, and plasma pyridoxine, cyanocobalamin, pteroylmonoglutamate and Cu concentrations were generally higher at the second time point, whilst plasma Zn and ascorbic acid concentrations were lower. ETK Ac was also generally lower at the second time point, which is indicative of an improvement in thiamin status. An overall effect of time was not observed for erythrocyte total thiamin, but a significant sex × time (Sex × Ti) interaction indicated an increase in thiamin concentration with time for the group of boys. The time effect, with respect to PCV, alkaline phosphatase activity and plasma cyanocobalamin, was greater for boys, while the time effect with respect to plasma Cu was greater for the girls. PCV, plasma alkaline phosphatase activity and plasma pteroylmonoglutamate concentration were significantly higher in the boys. A significant effect of supplementation (Tr) on plasma pteroylmonoglutamate, and a significant supplementation × time (Tr × Ti) effect on plasma cyanocobalamin was observed for both sexes.

Table 4. *Selected biochemical indices of mineral status for subjects aged 13–14 years before (1) and after (2) receiving a daily vitamin and mineral supplement (S) or a placebo (P)**

(Values are means with their standard errors with ranges in parentheses)

Treatment...	Boys								Girls							
	P (*n* 9)				S (*n* 10)				P (*n* 20)				S (*n* 12)			
	1		2		1		2		1		2		1		2	
Index	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Haemoglobin (Hb; g/l)	138	9	141	2	142	6	145	3	139	1	138	2	137	2	139	1
	(118–154)		(132–151)		(128–154)		(129–162)		(130–154)		(127–154)		(131–145)		(130–146)	
Packed cell volume (%)	40·1	0·3	45·2	0·8	41·5	0·3	44·7	1·3	40·8	0·5	42·6	0·7	39·5	0·4	43·1	0·4
	(35·2–44·3)		(43·3–48·2)		(38·5–44·2)		(40·3–49·6)		(37·5–44·7)		(40·3–48·0)		(37·8–42·0)		(40·2–44·5)	
Mean cell volume (fl)	83·2	0·9	87·6	1·9	82·0	1·2	84·4	1·0	84·3	0·6	86·7	1·1	83·4	1·0	87·2	1·2
	(79·7–89·3)		(81·3–94·5)		(77·0–90·3)		(81·0–88·0)		(77·3–88·3)		(79·7–90·0)		(77·3–89·5)		(83·0–95·5)	
Serum ferritin (μg/l)	23·1	3·2	23·2	1·8	24·7	2·9	30·1	6·0	20·8	2·7	19·6	2·1	21·1	5·2	24·8	3·6
	(8·5–40·0)		(16·0–30·0)		(8·2–40·0)		(10·0–67·0)		(2·0–51·5)		(6·0–41·0)		(6·5–56·0)		(8·0–44·0)	
Plasma Zn (μmol/l)	17·0	0·5	16·5	0·5	17·3	0·9	15·9	0·7	18·0	0·8	16·4	0·4	17·7	0·6	15·9	0·4
	(15·7–19·0)		(14·4–18·3)		(14·1–23·9)		(12·4–20·2)		(12·4–26·3)		(13·6–20·0)		(14·2–22·3)		(13·6–18·2)	
Plasma Cu (μmol/l)	12·5	1·1	14·1	0·8	13·3	0·9	14·9	0·8	12·1	0·7	15·9	0·9	12·0	0·7	14·4	0·7
	(9·4–15·5)		(12·1–18·9)		(9·4–18·2)		(10·5–18·8)		(7·6–19·7)		(9·9–27·9)		(8·0–15·7)		(12·1–18·4)	
Plasma alkaline phosphatase (*EC* 3.1.3.1; U/l)	68	7	110	20	80	11	91	8	48	5	45	5	34	4	38	3
	(43–105)		(58–222)		(51–146)		(55–137)		(21–97)		(20–93)		(15–62)		(24–60)	
Erythrocyte superoxide dismutase (*EC* 1.15.1.1; U/g Hb)	870	47	872	33	853	43	889	22	850	27	869	17	797	31	819	25
	(683–1132)		(650–954)		(673–1058)		(743–947)		(631–1130)		(735–1010)		(608–925)		(707–989)	
Whole-blood glutathione peroxidase (*EC* 1.11.1.9; U/mg Hb)	94	7	90	7	85	6	89	3	88	3	86	2	94	4	84	5
	(56–126)		(57–129)		(65–123)		(67–98)		(59–111)		(67–100)		(79–118)		(42–114)	

* For details of subjects and supplements, see Tables 1 and 2, p. 898 and pp. 902–904.

Table 5. *Selected biochemical indices of vitamin status for subjects aged 13–14 years before (1) and after (2) receiving a daily vitamin and mineral supplement (S) or a placebo (P)**

(Values are means with their standard errors with ranges in parentheses)

Treatment...	Boys								Girls							
	P (*n* 9)				S (*n* 10)				P (*n* 20)				S (*n* 12)			
	1		2		1		2		1		2		1		2	
Index	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma ascorbic acid (μmol/l)	72	7	58	7	88	14	73	15	80	4	65	3	68	6	58	3
	(40–98)		(20–76)		(39–208)		(32–207)		(44–113)		(43–91)		(37–99)		(37–75)	
Plasma pyridoxal-5-phosphate (nmol/l)	40	6	53	8	37	4	56	6	48	5	58	6	47	6	62	8
	(27–78)		(33–103)		(23–66)		(29–74)		(26–91)		(27–123)		(29–106)		(36–139)	
Plasma 25-hydroxy cholecalciferol (nmol/l)	67	10	65	5	64	5	75	9	66	6	74	7	76	7	65	8
	(35–107)		(40–121)		(36–82)		(32–127)		(26–120)		(32–137)		(29–117)		(22–118)	
Plasma cyanocobalamin (pmol/l)	337	38	589	101	323	37	978	147	363	28	522	49	352	33	652	59
	(178–451)		(281–1184)		(207–592)		(385–1628)		(192–681)		(215–1110)		(158–540)		(348–962)	
Plasma pteroylmonoglutamate (nmol/l)	26	2	29	5	26	4	42	4	22	1	22	2	20	2	31	3
	(20–32)		(10–50)		(16–47)		(19–50)		(14–33)		(10–43)		(12–33)		(18–50)	
Erythrocyte total thiamin (nmol/l)	184	18	212	29	227	28	257	58	203	10	172	13	224	28	193	9
	(120–252)		(113–403)		(128–446)		(112–627)		(103–307)		(85–321)		(101–492)		(141–246)	
ETK-Ac	1·11	0·07	0·95	0·05	1·01	0·04	1·06	0·03	1·08	0·04	1·03	0·02	1·11	0·03	0·97	0·02
	(0·69–1·36)		(0·70–1·24)		(0·81–1·26)		(0·90–1·20)		(0·86–1·45)		(0·84–1·21)		(0·87–1·27)		(0·88–1·11)	
EGR-Ac	1·28	0·10	1·25	0·04	1·20	0·07	1·22	0·04	1·14	0·03	1·23	0·02	1·22	0·04	1·23	0·03
	(1·02–1·78)		(1·10–1·44)		(1·00–1·65)		(1·03–1·38)		(1·00–1·52)		(1·03–1·46)		(1·01–1·47)		(1·03–1·46)	

ETK Ac, erythrocyte transketolase activity coefficient; EGR Ac, erythrocyte glutathione reductase activity coefficient.

* For details of subjects and supplements, see Tables 1 and 2, p. 898 and pp. 902–904.

Table 6. *Statistical* effect of sex (Sex), treatment (Tr) and time (Ti) on biochemical indices of micronutrient status*

Index	Sex	Tr	Ti	Sex × Tr	Sex × Ti	Tr × Ti	S × Tr × Ti
Mineral status							
Haemoglobin	—	—	—	—	—	—	—
Packed cell volume	< 0·05	—	< 0·01	—	< 0·01	—	< 0·05
Mean cell volume	—	—	< 0·001	—	—	—	< 0·05
Serum ferritin	—	—	—	—	—	—	—
Plasma zinc	—	—	< 0·01	—	—	—	—
Plasma copper	—	—	< 0·001	—	< 0·05	—	—
Plasma alkaline phosphatase (*EC* 3.1.3.1)†	< 0·001	—	< 0·01	—	< 0·01	—	< 0·05
Erythrocyte superoxide dismutase (*EC* 1.15.1.1)	—	—	—	—	—	—	—
Blood glutathione peroxidase (*EC* 1.11.1.9)	—	—	—	—	—	—	—
Vitamin status							
Plasma ascorbic acid	—	—	< 0·001	—	—	—	—
Plasma pyridoxal-5-phosphate†	—	—	< 0·001	—	—	—	—
Plasma 25-OH cholecalciferol	—	—	—	—	—	—	—
Plasma cyanocobalamin†	—	—	< 0·001	—	< 0·05	< 0·001	—
Plasma pteroylmonoglutamate	< 0·001	< 0·05	< 0·001	—	—	< 0·001	—
Erythrocyte total thiamin†	—	—	—	—	< 0·05	—	—
ETK-Ac	—	—	< 0·05	—	—	—	< 0·05
EGR-Ac	—	—	—	—	—	—	—

ETK-Ac, erythrocyte transketolase activity coefficient; EGR-Ac, erythrocyte glutathione reductase activity coefficient.

* Three-way analysis of variance; for details of statistical treatment, see p. 904.

† Data $\log_{10}$ transformed.

Effect of supplementation on verbal and non-verbal test scores

There was a significant retest effect (three-way analysis of variance) for all IQ scale scores (except vocabulary), but no effect of supplementation on any of the WISC-R Scales (Table 7).

The coding score was significantly higher in boys and there were significant Sex × Ti and Tr (placebo) × Ti interactions for digit span and picture completion respectively.

Although the study had a relatively small sample size (twenty-two supplemented subjects and twenty-nine placebo) a two-way analysis of variance (variables sex and treatment) of the change in total verbal score or total non-verbal score (see data in Table 7) indicated that with a residual 47 degrees of freedom and SED of means of 1·19 and 1·34 respectively, the smallest detectable significant increase in the effect of supplementation over and above the placebo effect (1-tail, $P > 0{\cdot}05$) would be 2·0 points for total verbal score and 2·3 points for total non-verbal score. Least significant differences of this magnitude do not seem unreasonably large when looking for any possible effect of supplementation.

Relationship between change in status and change in total verbal or non-verbal test score

The improvement in the value obtained for each biochemical index of status (excluding Hb, PCV and MCV) between the initial time point and 16 weeks later was calculated for each subject. The improvements in fourteen biochemical indices of status were ranked separately and, as a crude measure of 'overall' improvement in status with treatment, an average of

Table 7. *Test scores, and changes in test scores (D), for the WISC-R UK* administered individually before (1) and after (2) receiving a daily vitamin and mineral supplement (ten boys, twelve girls) or a placebo (nine boys, twenty girls) for 16 weeks†*

(Values are means with their standard errors‡)

Treatment...	Placebo						Supplement					
	1		2		D		1		2		D	
Scales	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Boys												
Information	10·9	0·9	12·0	0·9	1·1	0·5	10·9	0·7	11·6	0·6	0·7	0·2
Similarities	12·3	0·9	12·7	0·9	0·3	0·6	11·2	0·5	12·4	0·7	1·2	0·5
Vocabulary	12·9	1·2	12·9	1·0	0	0·4	13·1	0·6	13·7	0·8	0·6	0·4
Comprehension	12·6	0·6	14·0	1·1	1·4	0·7	11·9	0·8	12·7	0·8	0·8	0·6
Digit span	10·3	1·1	12·0	1·2	1·7	0·5	9·4	0·6	11·4	1·2	2·0	1·0
Verbal score	59·0	4·1	63·6	4·6	4·6	1·0	56·5	2·5	61·8	3·5	5·3	2·0
Picture completion	9·6	0·5	11·7	0·7	2·1	0·6	11·1	0·6	11·5	0·3	0·4	0·4
Picture arrangement	10·4	0·8	12·1	0·8	1·7	0·8	10·2	0·7	11·6	0·6	1·4	0·7
Block design	13·4	0·7	14·3	0·5	0·9	0·6	13·3	1·2	13·7	1·1	0·4	0·8
Object assembly	11·0	0·7	11·3	0·7	0·3	0·8	11·6	0·6	13·8	0·9	2·2	0·9
Coding	10·6	0·6	11·1	0·8	0·6	0·7	11·4	0·9	12·8	0·9	1·5	0·5
Non-verbal score	55·0	1·4	60·6	2·3	5·6	1·2	57·5	3·1	63·4	2·6	5·9	1·2
Girls												
Information	9·9	0·5	10·6	0·5	0·7	0·2	10·3	0·9	11·5	0·9	1·2	0·3
Similarities	11·6	0·4	12·7	0·4	1·1	0·3	10·8	0·5	12·3	0·6	1·6	0·5
Vocabulary	11·8	0·5	12·2	0·5	0·4	0·3	11·7	0·6	11·8	0·8	0·1	0·3
Comprehension	12·1	0·5	13·0	0·5	0·9	0·3	12·1	0·8	12·3	0·7	0·2	0·3
Digit span	11·3	0·8	11·1	0·2	0·2	0·5	10·2	0·9	11·1	0·6	0·9	0·8
Verbal score	56·7	2·1	59·6	2·2	2·9	0·7	55·0	3·1	59·1	3·0	4·1	1·2
Picture completion	10·1	0·5	11·7	0·4	1·6	0·3	9·3	0·5	10·6	0·5	1·3	0·5
Picture arrangement	10·6	0·4	12·6	0·4	2·0	0·4	11·8	0·5	13·3	0·5	1·5	0·8
Block design	13·8	0·5	14·4	0·7	0·6	0·5	13·1	0·7	13·8	0·8	0·7	0·6
Object assembly	12·0	0·5	12·3	0·7	0·3	0·7	12·3	0·9	13·8	1·0	1·5	1·0
Coding	13·5	0·5	14·7	0·6	1·2	0·4	12·0	0·8	12·8	1·0	0·8	0·4
Non-verbal score	60·0	1·1	65·6	1·7	5·7	1·2	58·4	2·1	64·4	2·6	6·0	1·4

* Wechsler Intelligence Scale for Children, Anglicized Revised Edition.

† For details of subjects and supplements, see Tables 1 and 2, p. 898 and pp. 902–904.

‡ Data were statistically examined using three-way analysis of variance for variables sex, treatment and time. For details of statistical analysis, see p. 904. There was a significant retest effect ($P < 0·05$) for all scale scores except vocabulary. There was a significant sex × time interaction for digit span ($P < 0·05$), a time × treatment interaction for picture completion ($P < 0·05$) and a sex effect for coding ($P < 0·01$).

ranks (average-rank) was calculated for each subject and then ranked once more (ranked average-rank). Ranked changes in either total verbal or total non-verbal IQ score over the period of treatment were not correlated (Spearman's non-parametric rank-correlation test) to this ranked average-rank of 'overall' change in status.

Compliance

Since supplementation resulted in a significant increase in plasma pteroylmonoglutamate and cyanocobalamin concentrations in the treatment group, these biochemical indices were examined in each individual allocated to this group. Four subjects were assessed as possible non-compliers, as judged by an absence of plasma pteroylmonoglutamate response and a

Table 8. *Relationships (R) between change in status with time (D) and initial status in groups of adolescents given a vitamin and mineral supplement (S) or a placebo (P)*†

(Values are means with their standard errors with ranges in parentheses)

Treatment...	P (*n* 29)				S (*n* 22)			
	D		R		D		R	
Index	Mean	SE	*r*	*P*	Mean	SE	*r*	*P*
Serum ferritin (μg/l)	−0·98 (−21·4–14·5)	1·74	−0·691	< 0·001	4·98 (−19–43)	3·60	−0·475	< 0·05
Plasma zinc (μmol/l)	−1·346 (−11·00–2·310)	0·673	−0·868	< 0·001	−1·484 (−7·357–3·410)	0·612	−0·722	< 0·001
Plasma copper (μmol/l)	3·305 (−1·621–8·341)	0·566	NS		2·061 (−3·619–6·294)	0·551	−0·552	< 0·05
Plasma alkaline phosphatase (*EC* 3.1.3.1; U/l)	8·30 (−34·5–117·0)	5·88	NS		7·02 (−38–36)	3·24	NS	
Erythrocyte superoxide dismutase (*EC* 1.15.1.1; U/g Hb)	8·4 (−263–251)	24·8	−0·797	< 0·001	23·0 (−185–235)	27·0	−0·759	< 0·001
Whole-blood glutathione peroxidase (*EC* 1.11.1.9; U/mg Hb)	−2·64 (−4·449–2·922)	3·36	−0·663	< 0·001	−3·70 (−3·859–3·273)	3·76	−0·644	< 0·01
Plasma ascorbic acid (μmol/l)	−15·79 (−53·95–11·36)	3·52	−0·565	< 0·01	−12·83 (−47·70–20·44)	3·75	NS	
Plasma pyridoxal-5-phosphate (nmol/l)	10·71 (−21·47–38·48)	2·90	NS		16·44 (−4·05–34·43)	2·93	NS	
Plasma 25-OH cholecalciferol (nmol/l)	4·69 (−67·14–86·61)	7·96	−0·730	< 0·001	−0·80 (−57·91–64·65)	6·89	−0·545	< 0·01
Plasma cyanocobalamin (pmol/l)	166 (−155–614)	37	NS		456*** (74–1199)	72	NS	
Plasma pteroylmonoglutamate (nmol/l)	0·261 (−19·75–23·61)	1·889	NS		13·71*** (−3·41–33·14)	2·61	NS	
Erythrocyte total thiamin (nmol/l)	−11·65 (−124·2–227·6)	15·42	−0·500	< 0·01	7·06 (−262·7–434·6)	27·93	−0·437	< 0·05
ETK-Ac	0·082 (−0·27–0·56)	0·046	+0·853	< 0·001	0·042 (−0·28–0·39)	0·039	+0·868	< 0·001
EGR-Ac	−0·044 (−0·34–0·57)	0·034	+0·870	< 0·001	−0·014 (−0·32–0·33)	0·035	+0·765	< 0·001

Hb, haemoglobin; ETK-Ac, erythrocyte transketolase activity coefficient; EGR-Ac, erythrocyte glutathione reductase activity coefficient; NS, not significant.

Mean change in status was significantly different from that of the placebo group; *** $P < 0·001$ (Student's unpaired *t* test).

† For details of subjects and supplements, see Tables 1 and 2, p. 898 and pp. 902–904.

smaller than expected increase in plasma cyanocobalamin concentration. Any values obtained for these individuals were removed from the database and all statistical analyses performed once more. The only difference observed between these and previous statistical analyses was a significant effect of supplementation on erythrocyte thiamin concentration.

Relationship between initial status and change in status with time

There was a significant degree of correlation in the placebo group between the change in many of the indices of status measured and the original status (Table 8). Most of these relationships were negative, with the highest improvement in status associated with the lowest initial status. The smallest improvement, or in most cases the largest decline, in status was associated with the highest initial status. Relationships for ETK-Ac and EGR-Ac were positive. However, since higher values for these two indices actually indicate a

lower status, these positive relationships also indicate an association between larger improvements in status when initial status was poorer.

Although supplementation had a tendency to lower the association between change in status and original status, in general responses were similar. However, the change in PAA relative to initial values was only significant ($P < 0{\cdot}01$) in the placebo group, whilst the change in plasma Cu relative to original status was significant ($P < 0{\cdot}05$) only in the supplemented group.

The change in either total verbal or total non-verbal score was not related to the initial total score in either placebo or supplemented groups.

Relationship between initial IQ score and initial status

There was no relationship between initial total verbal or total non-verbal score and initial status for most of the indices measured (all indices except Hb, PCV and MCV). In boys there were significant relationships between initial non-verbal score and PAA concentration (r 0·66; $P < 0{\cdot}001$), between initial non-verbal score and the joint effect of PAA and whole-blood glutathione peroxidase (GPX) activity (r 0·80; $P < 0{\cdot}001$), and between verbal score and GPX (r 0·8; $P < 0{\cdot}001$).

In girls there was a weak but significant relationship between initial non-verbal and verbal score and blood GPX (non-verbal, r 0·4, $P < 0{\cdot}05$; verbal, r 0·4, $P < 0{\cdot}05$).

DISCUSSION

Although overt manifestations of micronutrient deficiency have been largely eliminated in Western societies (Department of Health and Social Security, 1978), there may be a more common group of people in which a state of 'sub-optimal' nutrition exists. There has been considerable recent interest in the potential effects of 'sub-optimal' intakes of minerals and vitamins on psychological performance in schoolchildren, and controversy over whether vitamin and mineral supplementation could increase their non-verbal intelligence. Unfortunately, none of the recent studies that support such a hypothesis (Benton & Roberts, 1988; Benton & Buts, 1990; Schoenthaler *et al.* 1991*a*, *b*) has published any information on the nutritional status of their subjects either pre- or post-supplementation, and in some instances (Schoenthaler *et al.* 1991*a*, *b*) no information is presented on the dietary intake of micronutrients. Hence, the development of hypotheses in relation to diet–cognition interactions in children is extremely limited. Even where dietary information is provided, measurement of total amounts of specific micronutrients consumed may not provide a guide to status with respect to that nutrient, particularly in children where there is only limited information available on relationships between dietary intake and nutritional status, and requirements are poorly defined. The following discussion addresses these points in relation to the results obtained from the present study.

Reliability of intake recording

In the present study the method of recording diets every 6th day for 7 weeks was chosen in view of evidence that the accuracy of record keeping can deteriorate over two consecutive days' recording, and that usable records during days 5–7 are sometimes only obtained from more highly educated subjects (Gibson, 1990). There is also evidence that the added burden of duplicate diet collection can result in a decrease in recorded energy intakes by as much as 20% (Gibson, 1990). Average analysed energy intakes were around the estimated average requirement (EAR) for the age-group under study and have already been shown to be close to Food and Agriculture Organization/World Health Organization and Department of Health reports of estimates of expected daily energy expenditure (Finglas

et al. 1993). It appears, therefore, that there was no gross systematic under-recording of food intake. Comparison of calculated and analysed energy intakes indicates that duplicate diet collections were a good reflection of foods weighed and recorded in the dietary diary.

Calculated v. *analysed nutrient intakes*

Many studies, including recent investigations of the effect of micronutrient supplementation on intelligence, use food table data for the estimation of nutrient intake. One way of assessing the reliability of such data is to compare values computed from tables of food composition with values obtained by direct analysis of the diets consumed. It is clear from results presented here that there may be significant discrepancies between calculated and analysed intakes for some nutrients. Calculated values tended to underestimate thiamin and pteroylglutamate, and overestimate Cu, riboflavin and pyridoxine intakes. The microbiological assays used to construct the majority of food table data for pteroylglutamate and pyridoxine are now known to lack specificity and have been superseded by improved methods (Phillips & Wright, 1983; Polansky, 1985). Over the years there have also been improvements in the optimization of HPLC methods for thiamin and riboflavin, which may account for the discrepancies observed in the present study. In addition, since the intake of these two vitamins is heavily reliant on fortified foods, it is possible that in some cases the actual content of foods consumed may differ considerably from those analysed for the compilation of food tables. For the other micronutrients measured there appeared to be good agreement between 7 d mean calculated and analysed intakes. However, when the values are examined on an individual rather than a group basis there may be very marked differences between the two (Finglas *et al.* 1993). Therefore, when calculated intake values form the sole basis of nutritional assessment the potential inaccuracy of such values must be considered and discussed.

Micronutrient intake and status

The implication of recent studies reporting significant improvements in non-verbal IQ following micronutrient supplementation is that dietary deficiencies are hampering neural function in children. In those studies which presented dietary intake information, it may be tempting to assume that improvements in intelligence were associated with an increased intake of specific minerals and vitamins which appeared to be consumed in amounts less than recommended levels. It should be noted, however, that dietary reference values for children have not been established with any degree of confidence (Department of Health, 1991), because of the very limited information available on dietary requirements and on relationships between intake and biochemical indices of status in this age group. Reference limits and cut-off points for many biochemical indices are still a matter of debate, particularly for younger age groups where there is very little information available. The values presented in this paper, therefore, do not necessarily indicate a deficiency state, but are generally accepted as being at the lower end of the normal adult distribution (Gibson, 1990). One exception to this is the cut-off for abnormal serum ferritin concentration which has been suggested to be < 10 μg/l in children of 3–14 years, as compared with < 12 μg/l in older individuals (Dallman *et al.* 1980).

The relatively low concentrations of serum ferritin found in the present study (2–56 μg/l) are consistent with other findings of lower Fe stores in children of this age (Dallman *et al.* 1980). In the present study the proportion of boys with deficient Fe stores was similar to that observed in a National Health and Nutrition Examination Survey (NHANES II) carried out in the US (Fisher, 1985), but the incidence of Fe deficiency in the Norwich girls was considerably higher (21%) than that found in the much larger NHANES II study (6%).

Comparison of mean values for the range of status measurements performed in the present study with those measured in a fasting blood sample from adult subjects (*n* 160; eighty male, eighty female; 20–64 years) living in the same community (S. Southon, A. J. A. Wright, P. M. Finglas and A. L. Bailey, unpublished results) showed that values for plasma Zn, pyridoxine, and cyanocobalamin, EGR Ac and erythrocyte superoxide dismutase were similar. Plasma alkaline phosphatase activity was generally higher in adolescents, as expected (adolescents 62, adults 19 U/l), as was PAA concentration (adolescents 80, adults 48 μmol/l), which is discussed in detail elsewhere (Finglas *et al.* 1993). Plasma cholecalciferol concentrations in the 13–14-year-olds tended to be lower than those in adults, but none was below the lower adult cut-off limit of 17 nmol/l and comparisons are not strictly valid since the blood samples were taken in different seasons (adolescents 68, adults 195 nmol/l). Plasma pteroylmonoglutamate concentration in the adolescents tended to be higher than adults (adolescents 23, adults 15 nmol/l); this age-related trend has been noted elsewhere (Gibson, 1990). Although Cu concentration has been shown to rise to adult levels by 4 months of age (Gibson, 1990), values obtained for adolescents in the present study were lower than our adult subjects (adolescents 13, adults 17 μmol/l), with a substantial number of girls in particular having concentrations at the lower end of the normal adult range. The reasons for this are unclear, since analyses of samples for the two groups were performed at the same time. It was noted, however, that there was a significant increase in mean plasma Cu concentration with time in the girls.

Although values for erythrocyte thiamin concentration and ETK activity in adults are not available from our own study, comparisons with other published studies indicate that values for the adolescents were also at the bottom of the normal adult distribution (Brin, 1970; Warnock *et al.* 1978; Floridi *et al.* 1984; Baines, 1985; van der Westhuyzen *et al.* 1988).

In general, mean analysed intakes for both boys and girls were around, or well in excess of, the RNI value, apart from Fe and Zn intakes in the girls. On the basis of intake values alone, therefore, the diet could be considered as adequate. However, as discussed above, there were significant proportions of both boys and girls with 'low' biochemical indices of micronutrient status. It is recognized that some biochemical indices, although routinely used, are not particularly sensitive, and may be influenced by confounding factors unrelated to the dietary intake of the specific nutrient under investigation. However, even where biochemical measurements are known to provide a good indication of status, in an apparently healthy population, a relationship between total intake and status is not always found, because of the many dietary and physiological factors which influence absorption. A prime example of this is Fe (Southon *et al.* 1988).

Since there was little apparent relationship between intake and biochemical status, as judged by a range of indices, for most of the micronutrients examined, dietary information did not provide a useful prediction of potentially 'at risk' individuals. This highlights the importance of performing status measurements when considering the nutritional adequacy of the diet as a whole, and when drawing conclusions about the functional significance of variations in micronutrient intake.

A 4-month period of trace-element and vitamin (excluding A, E and K) supplementation, at RDA levels, appeared to have little impact upon the biochemical indices of micronutrient status measured in the present study, apart from plasma pteroylmonoglutamate and cyanocobalamin concentrations which each showed a significant increase after treatment. Although there were no other significant treatment effects, examination of the change in status with time (Table 8) indicated that there had been small increases in serum ferritin and erythrocyte thiamin concentrations in the supplemented group, whilst the mean

concentration in the placebo group had fallen. In addition, whilst there was a significant decline in PAA concentration in the placebo group, the change in PAA in the supplemented group did not reach significance.

The effects of time and initial status on the change in biochemical indices measured were more apparent. There was a significant effect of time on ten of the seventeen indices, and a significant correlation between the change in status compared with original status for nine out of fourteen indices examined in more detail. Care was taken to obtain both blood samples after a fasting period and at the same time of day to minimize diurnal variations. In addition, analyses were performed at a similar time point after sampling. The decrease in PAA observed in the present study is consistent with the finding of Widhalm *et al.* (1986), who observed that PAA declined with advancing adolescence, and it is known that MCV increases throughout childhood (Dallman *et al.* 1980). It is difficult to assess the implication of other observed changes in status with time, since there is little information on both short- and longer-term influences in this age group on the biochemical indices measured. However, since young children and adolescents experience several stages of rapid growth and development, it might be expected that this would have a marked influence on their dietary requirements and micronutrient status over relatively short periods. The results presented here indicate the importance of providing information on changes in status in investigations of the functional significance of micronutrient supplementation, not only for the test group but also for control and placebo groups. Observed changes in functional variables could then be assessed in relation to any changes in status, which may occur irrespective of the administration of dietary supplements. It would have been interesting, for example, to examine the relationship between changes in micronutrient status and performance in tests of intelligence in so called 'responders' in both the supplemented and placebo groups of the recent Schoenthaler study (Schoenthaler *et al.* 1991*b*).

Observations that the greatest improvement in status with time, with respect to several micronutrients, was associated with a lower initial status, and the smallest improvement (or largest decline) with a higher initial status, is entirely consistent with the hypothesis that subjects lower on the status curve would show a more sensitive response to positive status changes, whilst adaptive mechanisms would tend to resist change in a negative direction.

Micronutrients and IQ

No treatment effect was observed on either total verbal or total non-verbal test scores in the subjects under study although a difference of just 2·0 and 2·3 points respectively would have triggered a significant finding for the effects of supplementation. Estimation of 'overall' improvement of status for each subject over the study period, using an average-rank for the improvement in fourteen status indices, gave no significant relationship between change in 'overall' status and change in IQ. However, when status indices were examined individually there was a significant association between initial PAA concentration and non-verbal IQ in the boys, and whole-blood GPX activity and both non-verbal and verbal IQ in both sexes. A positive correlation between GPX and IQ has previously been shown for patients with Down's Syndrome (Sinet *et al.* 1979) and Se supplementation has recently been associated with significant alterations in profiles of mood (Benton & Cook, 1991), but no measurements of Se status were included in the study. In the present study GPX activity was used as an indicator of Se status, but this index is thought to be relatively insensitive, and is probably only indicative of a more pronounced deficiency state. It is recognized that the present observations, relating PAA and GPX to IQ scores, may well be chance findings, since false significances will inevitably increase with rising numbers of correlations performed. However, the provision of data which allow examination of such

relationships provides a better basis for the development of future research and facilitates comparison of any observed effects between studies.

In conclusion, although the present study used only a relatively small number of subjects compared with other recent studies in this area, the results highlight several important issues in relation to future investigations. First, it is likely that the use of food table data will lead to significant over or underestimation of the intake of several micronutrients. In any case the use of such data is questionable for the estimation of the intake of individuals, and should not be relied upon as the sole source of nutritional assessment. Second, in general the total calculated or analysed amount of a specific micronutrient consumed did not adequately predict the status of an individual, as judged by a range of biochemical indices, indicating the importance of status assessment in investigations of diet–function interactions. Third, nutritional status is dynamic and alters with dietary habits and physiological state. Studies involving younger individuals, in particular, should take account of possible changes in requirements and nutritional status, in both test and treatment groups, even over a relatively short study period. Results presented here have shown that changes in status with time are also markedly influenced by the initial status of the subject. Thus, ideally, allocation of subjects into test and treatment groups should include consideration of initial status indicators if possible. This is extremely difficult when studies are concerned with effects of a wide range of micronutrients, but is feasible if future investigations are more focused. Finally, although no effect of trace-element and vitamin (excluding A, E and K) supplementation on non-verbal intelligence was apparent in the present study, there was a significant association between two indices of status and initial IQ scores. These relationships may be fortuitous, but serve to illustrate that, until other studies include adequate nutritional assessment of subjects, comparison of such findings is not possible, and the development of hypotheses is limited.

This work was supported by the Ministry of Agriculture, Fisheries and Food, the Department of Education and Science, and the Vitamin Forum. The authors thank Joanne Belsten, Wendy Lee-Finglas, Jane Lumb, Zoe Piper and Heather Sarsby for their invaluable assistance with this study, and Dr Julia Leach for taking blood samples from the subjects. Our special thanks go to Mr Robert Tomson (educational psychologist) who conducted all the WISC-R UK tests, to the subjects themselves, their parents, teachers and the catering staff at the two schools.

REFERENCES

Anderson, S. H. & Nicol, A. D. (1986). A fluorometric method for measurement of erythrocyte transketolase activity. *Annals of Clinical Biochemistry* **23**, 180–189.

Bailey, A. L. & Finglas, P. M. (1990). A normal phase high-performance liquid chromatographic method for the determination of thiamin in blood and tissue samples. *Journal of Micronutrient Analysis* **7**, 147–157.

Baines, M. (1985). Improved HPLC detection of thiamin diphosphate in erythrocytes. *Clinica Chimica Acta* **153**, 43–48.

Behrens, W. A. & Madère, R. (1987). A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids and foods. *Analytical Biochemistry* **165**, 102–107.

Benton, D. & Buts, J.-P. (1990). Vitamin/mineral supplementation and intelligence. *Lancet* **335**, 1158–1160.

Benton, D. & Cook, R. (1991). The impact of selenium supplementation on mood. *Biological Psychiatry* **29**, 1092–1098.

Benton, D. & Roberts, G. (1988). Effect of vitamin and mineral supplementation on intelligence of a sample of schoolchildren. *Lancet* **i**, 140–143.

Bingham, S. A. (1987). The dietary assessment of individuals; methods, accuracy, new techniques and recommendations. *Nutrition Abstracts and Reviews (Series A)* **57**, 705–742.

Black, A. E., Ravenscroft, C. & Sims, A. J. (1984). The NACNE report; are the dietary goals realistic? Comparisons with the dietary patterns of dieticians. *Human Nutrition: Applied Nutrition* **38A**, 165–179.

Blinkhorn, S. (1991). A dose of vitamins and a pinch of salt. *Nature* **350**, 13.
Brin, M. (1970). Transketolase (sedoheptulose-7-phosphate: D-glyceral-dehyde-3-phosphate dihydroxyacetone transferase, *EC* 2.2.1.1) and the TPP effect in assessing thiamin status. In *Methods in Enzymology*, vol. 18 (part A), pp. 125–133 [D. B. McCormick and L. D. Wright, editors]. London: Academic Press.
Brubacher, G., Müller-Mulot, W. & Southgate, D. A. T. (editors) (1985). Vitamin B_6 in foodstuffs: HPLC method. In *Methods for the Determination of Vitamins in Food*, pp. 129–140. London: Elsevier Applied Sciences Publishers Ltd.
Crombie, I. K., Todman, J., McNeill, G., Florey, C. du V., Menzies, I. & Kennedy, R. A. (1990). Effect of vitamin and mineral supplementation on verbal and non-verbal reasoning of schoolchildren. *Lancet* **335**, 744–747.
Dallman, P. R., Siimes, M. A. & Stekel, A. (1980). Iron deficiency in infancy and childhood. *American Journal of Clinical Nutrition* **33**, 86–118.
Department of Health (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects* no. 41. London: H.M. Stationery Office.
Department of Health and Social Security (1978). *Prevention and Health*; *Eating for Health*. London: H.M. Stationery Office.
Department of Health and Social Security (1979). *Recommended Daily Amounts of Food Energy and Nutrients for Groups of People in the United Kingdom. Report on Health and Social Subjects* no. 15. London: H.M. Stationery Office.
De Souza, S. & Eitenmiller, R. (1990). Effects of different enzyme treatments on extraction of total folate from various foods prior to microbiological assay and radioassay. *Journal of Micronutrient Analysis* **7**, 37–57.
Eysenck, H. J. (1991). IQ and vitamin supplements. *Nature* **351**, 263.
Finglas, P. M., Bailey, A., Walker, A., Loughridge, J. L., Wright, A. J. A. & Southon, S. (1993). Vitamin C intake and plasma ascorbic acid concentration in adolescents. *British Journal of Nutrition* **69**, 563–576.
Fisher, K. D. (1985). Expert Scientific Working Group: summary of a report on assessment of the iron nutritional status of the United States population. *American Journal of Clinical Nutrition* **42**, 1318–1330.
Floridi, A., Pupita, M., Palmerini, C. A., Fini, C. & Fidanza, A. A. (1984). TPP retention in whole blood and erythrocytes by HPLC. *International Journal for Vitamin and Nutrition Research* **54**, 165–171.
Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972). Estimation of the concentration of the low density lipoprotein cholesterol in plasma without use of the preparative ultra centrifuge. *Clinical Chemistry* **18**, 499–502.
Gibson, R. S. (1990). *Principles of Nutritional Assessment*. Oxford, New York: Oxford University Press.
Herbert, V. (1966). Aseptic addition method for *Lactobacillus casei* assay of folate activity in human serum. *Journal of Clinical Pathology* **19**, 12–16.
Holland, B., Unwin, I. D. & Buss, D. H. (1988). *Cereals and Cereal Products. Third Supplement to McCance & Widdowson's The Composition of Foods*. Cambridge: RSC/MAFF.
Holland, B., Unwin, I. D. & Buss, D. H. (1989). *Milk Products and Eggs. Fourth Supplement to McCance & Widdowson's The Composition of Foods*. Cambridge RSC/MAFF.
Kwiatkowska, C. A., Finglas, P. M. & Faulks, R. M. (1989). The vitamin content of retail vegetables in the UK. *Journal of Human Nutrition and Dietetics* **2**, 159–172.
Lakshmaiah, N. & Ramasastri, B. V. (1980). Plasma folic acid conjugase. *Methods in Enzymology* **66**, 670–679.
Lancet letters (1988*a*). Vitamin/mineral supplementation and non-verbal intelligence. *Lancet* **i**, 407–409.
Lancet letters (1988*b*). Vitamins, minerals and IQ. *Lancet* **ii**, 744–745.
Miller, D. S. & Payne, P. R. (1959). A ballistic bomb calorimeter. *British Journal of Nutrition* **13**, 501–508.
Naoi, M. & Ichinose, H. (1988). Sensitive assay for determination of pyridoxal-5-phosphate in enzymes using high-performance liquid chromatography after derivatization with cyanide. *Journal of Chromatography* **434**, 209–214.
National Research Council (1980). *Recommended Dietary Allowances*, 9th ed. Washington DC: National Academy of Sciences.
Nelson, M., Naismith, D. J., Burley, V., Gatenby, S. & Geddes, N. (1990). Nutrient intakes, vitamin–mineral supplementation, and intelligence in British schoolchildren. *British Journal of Nutrition* **64**, 13–22.
Paglia, D. E. & Valentine, W. N. (1967). Studies on the quantitative and qualitative characteristics of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine* **70**, 158–169.
Paul, A. A. & Southgate, D. A. T. (1978). *McCance & Widdowson's The Composition of Foods*, 4th ed. London: H.M. Stationery Office.
Phillips, D. R. & Wright, A. J. A. (1982). Studies on the response of *Lactobacillus casei* to different folate monoglutamates. *British Journal of Nutrition* **47**, 183–189.
Phillips, D. R. & Wright, A. J. A. (1983). Studies on the response of *Lactobacillus casei* to folate vitamin in foods. *British Journal of Nutrition* **49**, 181–186.
Polansky, M. M., Reynolds, R. D. & Vanderslice, J. T. (1985). Vitamin B_6. In *Methods of Vitamin Analysis*, 4th ed., pp. 417–443 [J. Augustin, B. P. Klein, D. A. Becker and P. B. Venugopal, editors]. New York: John Wiley & Sons.
Powers, H. J., Bates, C. J., Prentice, A. M., Lamb, W. H., Jepson, M. & Bowman, H. (1983). The relative effectiveness of iron and iron with riboflavin in correcting microcytic anaemia in men and children in rural Gambia. *Human Nutrition: Clinical Nutrition* **37C**, 413–425.
Schoenthaler, S. J., Amos, S. P., Doraz, W. E., Kelly, M. A. & Wakefield J. Jr (1991*a*). Controlled trial of

vitamin–mineral supplementation on intelligence and brain function. *Personality and Individual Differences* **12**, 343–350.
Schoenthaler, S. J., Amos, S. P., Eysenck, H. J., Peritz, E. & Yudkin, J. (1991*b*). Controlled trial of vitamin–mineral supplementation: effects on intelligence and performance. *Personality and Individual Differences* **12**, 351–362.
Schrijver, J. (1991). Biochemical markers for micronutrient status and their interpretation. In *Modern Lifestyles, Lower Energy Intake and Micronutrient Status*, pp. 55–85. [K. Pietrzik editor]. London: Springer-Verlag.
Sinet, P.-M., Lejune, J. & Jerome, H. (1979). Trisomy 21 (Down's syndrome) glutathione peroxidase, hexose monophosphate shunt and IQ. *Life Sciences* **24**, 29–34.
Southon, S., Fairweather-Tait, S. J. & Hazell, T. (1988). Trace element availability from the human diet. *Proceedings of the Nutrition Society* **47**, 27–35.
Southon, S., Wright, A. J. A., Finglas, P. M., Bailey, A. L. & Belsten, J. L. (1992). Micronutrient intake and psychological performance of school children: consideration of the value of calculated nutrient intakes for the assessment of micronutrient status. *Proceedings of the Nutrition Society* **51**, 315–324.
Tamura, T. (1990). Microbiological assay of folates. In *Folic Acid Metabolism in Health and Disease*, pp. 121–137. [M. F. Picciano, E. L. R. Stokstad and J. F. Gregory III, editors]. New York: Wiley-Liss.
Tan, S. P., Wenlock, R. W. & Buss, D. H. (1985). *Immigrant Foods. Second Supplement to McCance & Widdowson's The Composition of Foods*. London: H.M. Stationery Office.
Thomas, B. (1988). *Manual of Dietetic Practice*, pp. 44–49. London: Scientific Publications.
van der Westhuyzen, J., Steyn, N. P., Icke, G. C. & Davis, R. E. (1988). Thiamin intakes and erythrocyte thiamin levels in eleven-year-old children in the Western Cape. *Tropical and Geographical Medicine* **40**, 218–222.
Warnock, L. G., Prudhomme, C. R. & Wagner, C. (1978). The determination of thiamin pyrophosphate in blood and other tissues and its correlation with erythrocyte transketolase activity. *Journal of Nutrition* **108**, 421–427.
Whanger, P. D., Beilstein, M. A., Thomson, C. D., Robinson, M. F. & Howe, M. (1988). Blood selenium and glutathione peroxidase activity of populations in New Zealand, Oregon and South Dakota. *FASEB Journal* **2**, 2996–3002.
Widhalm, K., Brubacher, G. & Christeller, S. (1986). Vitamin status of healthy adolescents 11 to 17 years old: data from a longitudinal study. *Monatsschrift für Kinderheilkunde* **134**, 408–414.
Wiles, S. J., Nettleton, P. A., Black, A. E. & Paul, A. A. (1980). The nutrient composition of some cooked dishes eaten in Britain: a supplementary food composition table. *Journal of Human Nutrition* **34**, 189–223.

Printed in Great Britain